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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07D 401/04, A61K 31/445 — A1

(11) International Publication Number: **WO 99/47512**

(43) International Publication Date: 23 September 1999 (23.09.99)

(21) International Application Number: PCT/US99/05562

(22) International Filing Date: 16 March 1999 (16.03.99)

(30) Priority Data:
60/078,180 16 March 1998 (16.03.98) US

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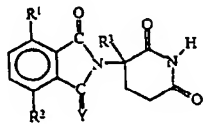
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: 2-(2,6-DIOXOPIPERIDIN-3-YL)ISOINDOLINE DERIVATIVES, THEIR PREPARATION AND THEIR USE AS INHIBITORS OF INFLAMMATORY CYTOKINES

(57) Abstract

1-Oxo- and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)isoindolines of formula (I) in which Y is oxygen or H_2, one of R^1 and R^2 is halo, alkyl, alkoxy, alkylamino, dialkylamino, cyano, or carbamoyl, the other of R^1 and R^2 is independently hydrogen, halo, alkyl, alkoxy, alkylamino, dialkylamino, cyano, or carbamoyl and; R^3 is hydrogen, alkyl, or benzyl, and the method of reducing levels of tumor necrosis factor α and other inflammatory cytokines in a mammal through the administration of such derivatives, and pharmaceutical compositions containing such derivatives.



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2-(2,6-DIOXOPIPERIDIN-3-YL)ISOINDOLINE DERIVATIVES, THEIR PREPARATION AND THEIR USE AS INHIBITORS OF INFLAMMATORY CYTOKINES

This application claims the benefit of U.S. Provisional Application No. 60/078,180 filed on 3/16/98 entitled 1-Oxo- and 1,3-Dioxoisoindolines and Method of Reducing Inflammatory Cytokine Levels, hereby incorporated by reference into this application.

Background of the Invention

Tumor necrosis factor-α, or TNFα, is a cytokine which is released primarily by mononuclear phagocytes in response to a number immunostimulators. It is a key proinflammatory cytokine in the inflammation cascade causing the production and/or release of other cytokines and agents. When administered to animals or humans, it causes inflammation, fever, cardiovascular effects, hemorrhage, coagulation, and acute phase responses similar to those seen during acute infections and shock states. Excessive or unregulated TNFα production thus has been implicated in a number of disease conditions. These include endotoxemia and/or toxic shock syndrome {Tracey *et al.*, *Nature* **330**, 662-664 (1987) and Hinshaw *et al.*, *Circ. Shock* **30**, 279-292 (1990)}; cachexia {Dezube *et al.*, *Lancet*, **335** (8690), 662 (1990)} and Adult Respiratory Distress Syndrome (ARDS) where TNFα concentration in excess of 12,000 pg/mL have been detected in pulmonary aspirates from ARDS patients {Millar *et al.*, *Lancet* **2**(8665), 712-714 (1989)}. Systemic infusion of recombinant TNFα also resulted in changes typically seen in ARDS {Ferrai-Baliviera *et al.*, *Arch. Surg.* **124**(12), 1400-1405 (1989)}.

TNFα appears to be involved in bone resorption diseases, including arthritis. When activated, leukocytes will produce bone-resorption, an activity to which the data suggest TNFα contributes. {Bertolini *et al.*, *Nature* **319**, 516-518 (1986) and Johnson *et al.*, *Endocrinology* **124**(3), 1424-1427 (1989).} TNFα also has been shown to stimulate bone resorption and inhibit bone formation *in vitro* and *in vivo* through stimulation of osteo-

clast formation and activation combined with inhibition of osteoblast function. Although TNFα may be involved in many bone resorption diseases, including arthritis, the most compelling link with disease is the association between production of TNFα by tumor or host tissues and malignancy associated hypercalcemia {*Calci. Tissue Int. (US)* **46**(Suppl.), S3-10 (1990)}. In Graft versus Host Reaction, increased serum TNFα levels have been associated with major complication following acute allogenic bone marrow transplants {Holler *et al.*, *Blood*, **75**(4), 1011-1016 (1990)}.

Cerebral malaria is a lethal hyperacute neurological syndrome associated with high blood levels of TNFα and the most severe complication occurring in malaria patients. Levels of serum TNFα correlated directly with the severity of disease and the prognosis in patients with acute malaria attacks {Grau *et al.*, *N. Engl. J. Med.* **320**(24), 1586-1591 (1989)}.

Macrophage-induced angiogenesis is known to be mediated by TNFα. Leibovich *et al.* {*Nature*, **329**, 630-632 (1987)} showed TNFα induces *in vivo* capillary blood vessel formation in the rat cornea and the developing chick chorioallantoic membranes at very low doses and suggest TNFα is a candidate for inducing angiogenesis in inflammation, wound repair, and tumor growth. TNFα production also has been associated with cancerous conditions, particularly induced tumors {Ching *et al.*, *Brit. J. Cancer*, (1955) **72**, 339-343, and Koch, *Progress in Medicinal Chemistry*, **22**, 166-242 (1985)}.

TNFα also plays a role in the area of chronic pulmonary inflammatory diseases. The deposition of silica particles leads to silicosis, a disease of progressive respiratory failure caused by a fibrotic reaction. Antibody to TNFα completely blocked the silica-induced lung fibrosis in mice {Pignet *et al.*, *Nature*, **344**, 245-247 (1990)}. High levels of TNFα production (in the serum and in isolated macrophages) have been demonstrated in animal models of silica and asbestos induced fibrosis {Bissonnette *et al.*, *Inflammation* **13**(3), 329-339 (1989)}. Alveolar macrophages from pulmonary sarcoidosis patients have also

been found to spontaneously release massive quantities of TNFα as compared with macrophages from normal donors {Baughman *et al.*, *J. Lab. Clin. Med.* **115**(1), 36-42 (1990)}.

TNFα is also implicated in the inflammatory response which follows reperfusion, called reperfusion injury, and is a major cause of tissue damage after loss of blood flow {Vedder *et al.*, *PNAS* **87**, 2643-2646 (1990)}. TNFα also alters the properties of endothelial cells and has various pro-coagulant activities, such as producing an increase in tissue factor pro-coagulant activity and suppression of the anticoagulant protein C pathway as well as down-regulating the expression of thrombomodulin {Sherry *et al.*, *J. Cell Biol.* **107**, 1269-1277 (1988)}. TNFα has pro-inflammatory activities which together with its early production (during the initial stage of an inflammatory event) make it a likely mediator of tissue injury in several important disorders including but not limited to, myocardial infarction, stroke and circulatory shock. Of specific importance may be TNFα-induced expression of adhesion molecules, such as intercellular adhesion molecule (ICAM) or endothelial leukocyte adhesion molecule (ELAM) on endothelial cells {Munro *et al.*, *Am. J. Path.* **135**(1), 121-132 (1989)}.

TNFα blockage with monoclonal anti-TNFα antibodies has been shown to be beneficial in rheumatoid arthritis {Elliot *et al.*, *Int. J. Pharmac.* 1995 **17**(2), 141-145}. High levels of TNFα are associated with Crohn's disease {von Dullemen *et al.*, *Gastroenterology*, 1995 **109**(1), 129-135} and clinical benefit has been achieved with TNFα antibody treatment.

Moreover, it now is known that TNFα is a potent activator of retrovirus replication including activation of HIV-1. {Duh *et al.*, *Proc. Nat. Acad. Sci.* **86**, 5974-5978 (1989); Poll *et al.*, *Proc. Nat. Acad. Sci.* **87**, 782-785 (1990); Monto *et al.*, *Blood* **79**, 2670 (1990); Clouse *et al.*, *J. Immunol.* **142**, 431-438 (1989); Poll *et al.*, *AIDS Res. Hum. Retrovirus*, 191-197 (1992)}. AIDS results from the infection of T lymphocytes with

Human Immunodeficiency Virus (HIV). At least three types or strains of HIV have been identified, *i.e.*, HIV-1, HIV-2 and HIV-3. As a consequence of HIV infection, T-cell mediated immunity is impaired and infected individuals manifest severe opportunistic infections and/or unusual neoplasms. HIV entry into the T lymphocyte requires T lymphocyte activation. Other viruses, such as HIV-1, HIV-2 infect T lymphocytes after T cell activation and such virus protein expression and/or replication is mediated or maintained by such T cell activation. Once an activated T lymphocyte is infected with HIV, the T lymphocyte must continue to be maintained in an activated state to permit HIV gene expression and/or HIV replication. Cytokines, specifically TNFα, are implicated in activated T-cell mediated HIV protein expression and/or virus replication by playing a role in maintaining T lymphocyte activation. Therefore, interference with cytokine activity such as by prevention or inhibition of cytokine production, notably TNFα, in an HIV-infected individual assists in limiting the maintenance of T lymphocyte caused by HIV infection.

Monocytes, macrophages, and related cells, such as kupffer and glial cells, also have been implicated in maintenance of the HIV infection. These cells, like T cells, are targets for viral replication and the level of viral replication is dependent upon the activation state of the cells. {Rosenberg *et al.*, *The Immunopathogenesis of HIV Infection*, Advances in Immunology, **57** (1989)}. Cytokines, such as TNFα, have been shown to activate HIV replication in monocytes and/or macrophages {Poli *et al.*, *Proc. Natl. Acad. Sci.*, **87**, 782-784 (1990)}, therefore, prevention or inhibition of cytokine production or activity aids in limiting HIV progression for T cells. Additional studies have identified TNFα as a common factor in the activation of HIV *in vitro* and has provided a clear mechanism of action via a nuclear regulatory protein found in the cytoplasm of cells (Osborn, *et al.*, *PNAS* **86** 2336-2340). This evidence suggests that a reduction of TNFα synthesis may have an antiviral effect in HIV infections, by reducing the transcription and thus virus production.

AIDS viral replication of latent HIV in T cell and macrophage lines can be induced by TNFα {Folks *et al.*, *PNAS* **86**, 2365-2368 (1989)}. A molecular mechanism for the virus inducing activity is suggested by TNFα's ability to activate a gene regulatory protein (NFκB) found in the cytoplasm of cells, which promotes HIV replication through binding to a viral regulatory gene sequence (LTR) {Osborn *et al.*, *PNAS* **86**, 2336-2340 (1989)}. TNFα in AIDS associated cachexia is suggested by elevated serum TNFα and high levels of spontaneous TNFα production in peripheral blood monocytes from patients {Wright *et al.*, *J. Immunol.* **141**(1), 99-104 (1988)}. TNFα has been implicated in various roles with other viral infections, such as the cytomegalia virus (CMV), influenza virus, adenovirus, and the herpes family of viruses for similar reasons as those noted.

The nuclear factor κB (NFκB) is a pleiotropic transcriptional activator (Lenardo, *et al.*, *Cell* 1989, 58, 227-29). NFκB has been implicated as a transcriptional activator in a variety of disease and inflammatory states and is thought to regulate cytokine levels including but not limited to TNFα and also to be an activator of HIV transcription (Dbaibo, *et al.*, *J. Biol. Chem.* 1993, 17762-66; Duh *et al.*, *Proc. Natl. Acad. Sci.* 1989, 86, 5974-78; Bachelerie *et al.*, *Nature* 1991, 350, 709-12; Boswas *et al.*, *J. Acquired Immune Deficiency Syndrome* 1993, 6, 778-786; Suzuki *et al.*, *Biochem. And Biophys. Res. Comm.* 1993, 193, 277-83; Suzuki *et al.*, *Biochem. And Biophys. Res Comm.* 1992, 189, 1709-15; Suzuki *et al.*, *Biochem. Mol. Bio. Int.* 1993, 31(4), 693-700; Shakhov *et al.*, *Proc. Natl. Acad. Sci. USA* 1990, 171, 35-47; and Staal *et al.*, *Proc. Natl. Acad. Sci. USA* 1990, 87, 9943-47). Thus, inhibition of NFκB binding can regulate transcription of cytokine gene(s) and through this modulation and other mechanisms be useful in the inhibition of a multitude of disease states. The compounds described herein can inhibit the action of NFκB in the nucleus and thus are useful in the treatment of a variety of diseases including but not limited to rheumatoid arthritis, rheumatoid spondylitis, osteo-arthritis, other arthritic conditions, septic shock, septis, endotoxic shock, graft versus host

disease, wasting, Crohn's disease, ulcerative colitis, multiple sclerosis, systemic lupus erythrematosis, ENL in leprosy, HIV, AIDS, and opportunistic infections in AIDS. TNFα and NFκB levels are influenced by a reciprocal feedback loop. As noted above, the compounds of the present invention affect the levels of both TNFα and NFκB.

Many cellular functions are mediated by levels of adenosine 3',5'-cyclic monophosphate (cAMP). Such cellular functions can contribute to inflammatory conditions and diseases including asthma, inflammation, and other conditions (Lowe and Cheng, *Drugs of the Future*, *17*(9), 799-807, 1992). It has been shown that the elevation of cAMP in inflammatory leukocytes inhibits their activation and the subsequent release of inflammatory mediators, including TNFα and NFκB. Increased levels of cAMP also leads to the relaxation of airway smooth muscle. Phosphodiesterases control the level of cAMP through hydrolysis and inhibitors of phosphodiesterases have been shown to increase cAMP levels.

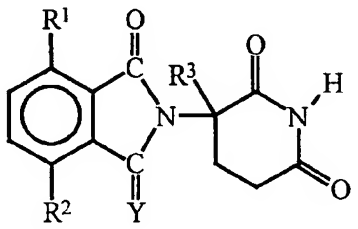
Decreasing TNFα levels and/or increasing cAMP levels thus constitutes a valuable therapeutic strategy for the treatment of many inflammatory, infectious, immunological or malignant diseases. These include but are not restricted to septic shock, sepsis, endotoxic shock, hemodynamic shock and sepsis syndrome, post ischemic reperfusion injury, malaria, mycobacterial infection, meningitis, psoriasis, congestive heart failure, fibrotic disease, cachexia, graft rejection, cancer, autoimmune disease, opportunistic infections in AIDS, rheumatoid arthritis, rheumatoid spondylitis, osteoarthritis, other arthritic conditions, Crohn's disease, ulcerative colitis, multiple sclerosis, systemic lupus erythrematosis, ENL in leprosy, radiation damage, and hyperoxic alveolar injury. Prior efforts directed to the suppression of the effects of TNFα have ranged from the utilization of steroids such as dexamethasone and prednisolone to the use of both polyclonal and monoclonal antibodies {Beutler *et al.*, *Science* **234**, 470-474 (1985); WO 92/11383}.

Detailed Description

The present invention is based on the discovery that certain classes of non-polypeptide compounds more fully described herein decrease the levels of TNFα, increase cAMP levels, and inhibit inflammatory cytokines. The present invention thus relates to 1-oxo- and 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)isoindolines substituted in the 4-position of the isoindoline ring and optionally further substituted in the 3-position of the 2,6-dioxopiperidine ring , the method of reducing levels of tumor necrosis factor α and other inflammatory cytokines in a mammal through the administration of such derivatives, and pharmaceutical compositions containing such derivatives.

In particular, the invention pertains to

(*a*) a 2-(2,6-dioxopiperidin-3-yl)-isoindoline of the formula:



I.

in which

Y is oxygen or H_2,

a first of R^1 and R^2 is halo, alkyl, alkoxy, alkylamino, dialkylamino, cyano, or carbamoyl,

the second of R^1 and R^2, independently of the first, is hydrogen, halo, alkyl, alkoxy, alkylamino, dialkylamino, cyano, or carbamoyl, and

R^3 is hydrogen, alkyl, or benzyl, and

(*b*) the acid addition salts of said 2-(2,6-dioxopiperidin-3-yl)-isoindolines which contain a nitrogen atom capable of being protonated.

Unless otherwise defined, the term alkyl denotes a univalent saturated branched or straight hydrocarbon chain containing from 1 to 4 carbon atoms. Representative of such alkyl groups are methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, and tert-butyl. Alkoxy refers to an alkyl group bound to the remainder of the molecule through an ethereal oxygen atom. Representative of such alkoxy groups are methoxy, ethoxy, propoxy, isopropoxy, butoxy, isobutoxy, sec-butoxy, and tert-butoxy.

Halo includes bromo, chloro, fluoro, and iodo.

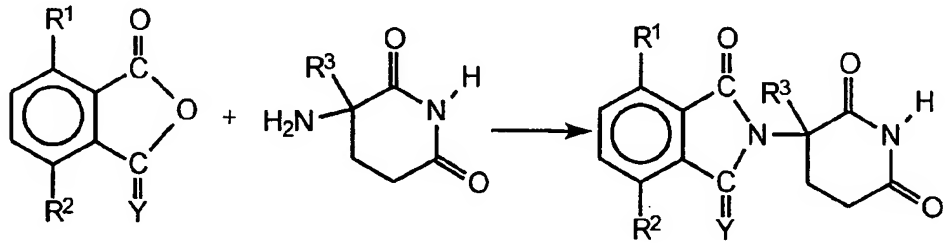
The compounds of Formula I are used, under the supervision of qualified professionals, to inhibit the undesirable effects of TNFα and other inflammatory cytokines including the interleukins IL-1, IL-6, and IL-12. The compounds can be administered orally, rectally, or parenterally, alone or in combination with other therapeutic agents including antibiotics, steroids, chemotherapeutic agents, *etc.*, to a mammal in need of treatment; *e.g.*, in the treatment of cancers, rheumatoid arthritis, inflammatory bowel disease, muscular dystrophy, Crohn's disease, *etc.*.

The compounds of the present invention also can be used topically in the treatment or prophylaxis of disease states mediated or exacerbated by excessive TNFα production, respectively, such as viral infections, such as those caused by the herpes viruses, or viral conjunctivitis, psoriasis, atopic dermatitis, *etc.*

The compounds also can be used in the veterinary treatment of mammals other than humans in need of prevention or inhibition of TNFα production. TNFα mediated diseases for treatment, therapeutically or prophylactically, in animals include disease states such as those noted above, but in particular viral infections. Examples include

feline immunodeficiency virus, equine infectious anaemia virus, caprine arthritis virus, visna virus, and maedi virus, as well as other lentiviruses.

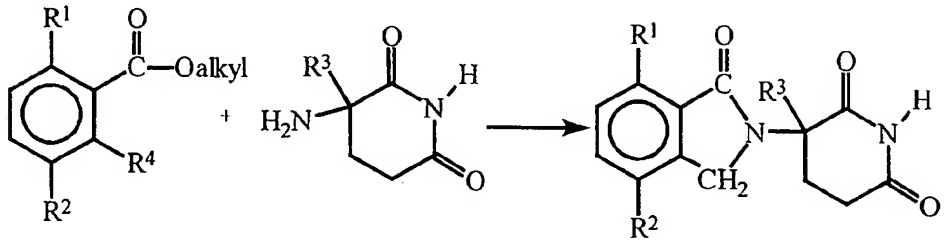
The compounds of Formula I are readily prepared through a number of routes. In a first embodiment, an anhydride or lactone is allowed to react with a 3-amino-2,6-dioxo-piperidine:



In the foregoing reactions, each of R^1, R^2, R^3, and Y are as defined above.

The 3-amino-2,6-dioxopiperidine can be obtained from the corresponding glutamic acid anhydride through conventional amidation or from the cyclization of appropriate glutamine derivatives..

The compounds in which Y is H_2 alternatively can be obtained from a disubstituted benzoate intermediate according to the following reactions:

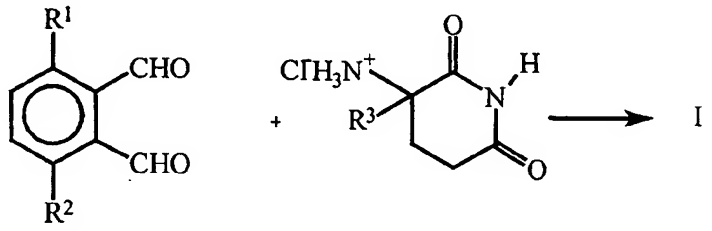


in which R^4 is CHO or CH_2Br in the presence of an acid acceptor such as dimethylaminopyridine or triethylamine.

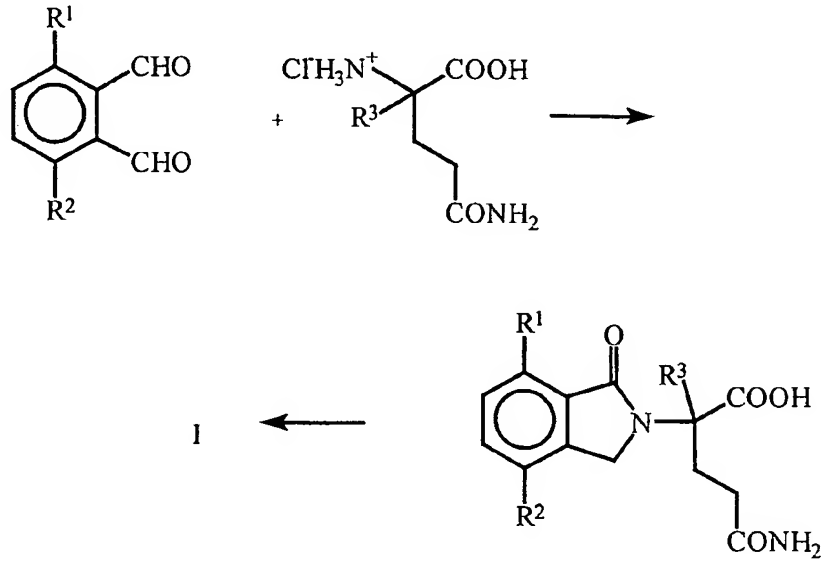
The disubstituted benzoate intermediates are known or can be obtained though conventional processes. For example, a lower alkyl ester of a 3,6-disubstituted *ortho*-toluic

acid is brominated with N-bromosuccinimide under the influence of light to yield the lower alkyl 2-(bromomethyl)-3,6-disubstitutedbenzoate.

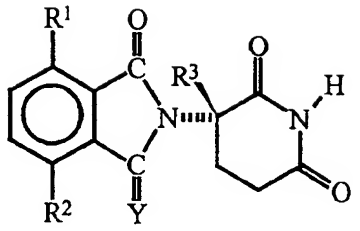
Alternatively, a dialdehyde is allowed to react with 2,6-dioxopiperidin-3-ammonium chloride to obtain the compounds of Formula I in which Y is H_2:

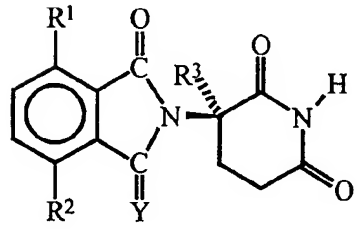


Finally, a dialdehyde is allowed to react with glutamine and the resulting 2-(1-oxo-isoindolin-2-yl)glutaric acid then cyclized to yield a 4,7-disubstituted 1-oxo-2-(2,6-dioxo-piperidin-3-yl)-isoindoline of Formula I in which Y is H_2:



The carbon atom to which R^3 is bound in the compounds of Formula I constitutes a center of chirality, thereby giving rise to optical isomers:





Both the racemates of these isomers and the individual isomers themselves, as well as diastereomers when a second chiral center is present, are within the scope of the present invention. The racemates can be used as such or can be separated into their individual isomers mechanically as by chromatography using a chiral absorbent. Alternatively, the individual isomers can be prepared in chiral form or separated chemically from a mixture by forming salts with a chiral acid or base, such as the individual enantiomers of 10-camphorsulfonic acid, camphoric acid, α-bromocamphoric acid, methoxyacetic acid, tartaric acid, diacetyltartaric acid, malic acid, pyrrolidone-5-carboxylic acid, and the like, and then freeing one or both of the resolved bases, optionally repeating the process, so as obtain either or both substantially free of the other; *i.e.*, in a form having an optical purity of >95%.

The present invention also pertains to the physiologically acceptable non-toxic acid addition salts of the compound of Formula I which contain a group capable of being protonated; *e.g.*, amino. Such salts include those derived from organic and inorganic acids such as, without limitation, hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, methanesulphonic acid, acetic acid, tartaric acid, lactic acid, succinic acid,

citric acid, malic acid, maleic acid, sorbic acid, aconitic acid, salicylic acid, phthalic acid, embonic acid, enanthic acid, and the like.

Particularly preferred compounds include 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-methylisoindoline, 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-ethylisoindoline, 1,3-dioxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4-methylisoindoline, 1,3-dioxo-2-(2,6-dioxoopiperidin-3-yl)-4,7-dimethylisoindoline, 1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4-ethylisoindoline, 1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4-methylisoindoline, 1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-7-ethylisoindoline, 1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-7-methylisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-propylisoindoline, 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-chloroisoindoline, 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-carbamoylisoindoline, 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-methoxyisoindoline, 1-oxo-2-(2,6-dioxoopiperidin-3-yl)-4,7-dimethylisoindoline, 1-oxo-2-(2,6-dioxoopiperidin-3-yl)-4-methyl-7-ethylisoindoline, and 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,7-diethoxyisoindoline. Of these, 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-methylisoindoline, 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4,7-dimethylisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-methylisoindoline, and 1 oxo-2-(2,6-dioxopiperidin-3-yl)-4,7-dimethylisoindoline are particularly preferred.

Oral dosage forms include tablets, capsules, dragees, and similar shaped, compressed pharmaceutical forms containing from 1 to 100 mg of drug per unit dosage. Isotonic saline solutions containing from 20 to 100 mg/mL can be used for parenteral administration which includes intramuscular, intrathecal, intravenous and intra-arterial routes of administration. Rectal administration can be effected through the use of suppositories formulated from conventional carriers such as cocoa butter.

Pharmaceutical compositions thus comprise one or more compounds of Formulas I associated with at least one pharmaceutically acceptable carrier, diluent or excipient. In preparing such compositions, the active ingredients are usually mixed with or diluted by

an excipient or enclosed within such a carrier which can be in the form of a capsule or sachet. When the excipient serves as a diluent, it may be a solid, semi-solid, or liquid material which acts as a vehicle, carrier, or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, elixirs, suspensions, emulsions, solutions, syrups, soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders. Examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starch, gum acacia, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidinone, cellulose, water, syrup, and methyl cellulose, the formulations can additionally include lubricating agents such as talc, magnesium stearate and mineral oil, wetting agents, emulsifying and suspending agents, preserving agents such as methyl- and propylhydroxybenzoates, sweetening agents or flavoring agents.

The compositions preferably are formulated in unit dosage form, meaning physically discrete units suitable as a unitary dosage, or a predetermined fraction of a unitary dose to be administered in a single or multiple dosage regimen to human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with a suitable pharmaceutical excipient. The compositions can be formulated so as to provide an immediate, sustained or delayed release of active ingredient after administration to the patient by employing procedures well known in the art.

Enzyme-linked immunosorbent assays for TNFα can be performed in a conventional manner. PBMC is isolated from normal donors by Ficoll-Hypaque density centrifugation. Cells are cultured in RPMI supplemented with 10% AB+ serum, 2mM L-glutamine, 100 U/mL penicillin, and 100 mg/mL streptomycin. Drugs are dissolved in dimethylsulfoxide (Sigma Chemical) and further dilutions are done in supplemented RPMI. The final dimethylsulfoxide concentration in the presence or absence of drug in the PBMC suspensions is 0.25 wt %. Drugs are assayed at half-log dilutions starting at 50 mg/mL.

Drugs are added to PBMC (10^6 cells/mL) in 96 wells plates one hour before the addition of LPS. PBMC (10^6 cells/mL) in the presence or absence of drug are stimulated by treatment with 1 mg/mL of LPS from *Salmonella minnesota* R595 (List Biological Labs, Campbell, CA). Cells are then incubated at 37° C for 18-20 hours. Supernatants are harvested and assayed immediately for TNFα levels or kept frozen at -70°C (for not more than 4 days) until assayed. The concentration of TNFα in the supernatant is determined by human TNFα ELISA kits (ENDOGEN, Boston, MA) according to the manufacturer's directions.

The following examples will serve to further typify the nature of this invention but should not be construed as a limitation in the scope thereof, which scope is defined solely by the appended claims.

EXAMPLE 1

2-(2,6-Dioxopiperid-3-yl)-4-methylisoindoline-1,3-dione

A stirred solution of 3-methylphthalic anhydride (2.96 g, 18.2 mmol), 3-aminopiperidine2,6-dione hydrogen chloride (3.00 g, 18.2 mmol) and sodium acetate (1.57 g, 19.1 mmol) in acetic acid (30 mL) was heated at reflux for 23 hours. The solvent was removed *in vacuo* to give a solid which was stirred with water (40 mL) for 1 hour, filtered, washed with water (30 mL), and then heated with decolorizing charcoal (1 g) in acetone (2 L) at reflux temperature for 30 min. The suspension was filtered through a pad of Celite to give a clear solution. The solvent of filtrate was removed *in vacuo* to give 2-(2,6-dioxopiperid-3-yl)-4-methylisoindoline-1,3-dione as a white solid (4.08 g, 82 % yield)- mp 290.0-292.0 °C; ^{1}H NMR (DMSO-d6); δ 2.03-2.09 (m, 1H, CH*H*), 2.50-2.60 (m, 2H, CH_2), 2.63 (s, 3H, CH_3), 2.83-2.95 (m, IH, CH*H*), 5.13 (dd, J = 5.4,12.3 Hz, IH, NC*H*), 7.65-7.79 (m, 3H, Ar), 11.13 (br s, IH, N*H)*; ^{13}C NMR (DMSO-d6) δ 17.04, 21.99, 30.93, 48.76, 121.05, 127.89, 131.63, 134.37, 136.91, 137.61, 167.04, 167.83, 169.87, 172.74; Anal Calcd for $C_{14}H_{12}N_2O_4$: C, 61.76; H, 4.44; N, 10.29. Found: C, 61.68; H, 4.3 7; N, 10.17.

EXAMPLE 2

By substituting equivalent amounts of 3-ethylphthalic anhydride, 3-fluorophthalic anhydride, 3-chlorophthalic anhydride, 3-carbamoylphthalic anhydride, and 3-methoxyphthalic anhydride in the procedure of Example 1, there are respectively obtained 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-ethylisoindoline, 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-fluoroisoindoline, 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-chloroisoindoline, 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-carbamoylisoindoline, 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-methoxyisoindoline.

EXAMPLE 3

By substituting equivalent amounts of 3-amino-3-methylpiperidine2,6-dione hydrogen chloride for 3-aminopiperidine2,6-dione hydrogen chloride in the procedure of Example 1, 1,3-dioxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4-methylisoindoline is obtained.

EXAMPLE 4

1-Oxo-2-(2,6-dioxopiperidin-3-yl)-4-methylisoindoline

A mixture of 16.25 g of 2,6-dioxopiperidin-3-ammonium chloride, and 30.1 g of methyl 2-bromomethyl-3-methylbenzoate, and 12.5 g of triethylamine in 100 mL of dimethylformamide is stirred at room temperature for 15 hours. The mixture is then concentrated *in vacuo* and the residue mixed with methylene chloride and water. The aqueous layer is separated and back-extracted with methylene chloride. The combined methylene chloride solutions are dried over magnesium sulfate and concentrated *in vacuo* to give 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-methylisoindoline.

In a similar fashion 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,7-dimethylisoindoline, 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-ethylisoindoline, and 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-methoxyisoindoline are obtained by substituting equivalent amounts of methyl 2-bromomethyl-3,6-dimethylbenzoate, methyl 2-bromomethyl-3-ethylbenzoate, and methyl

2-bromomethyl-3-methoxybenzoate, respectively, for methyl 2-bromomethyl-3-methylbenzoate.

EXAMPLE 5

2-(2,6-Dioxopiperidin-3-yl)-4,7-dimethylisoindoline-1,3-dione

2-(2,6-Dioxopiperid-3-yl)-4,7-dimethylisoindoline-1,3-dione was prepared by the procedure of Example 1 from 3,6-dimethylphthalic anhydride (220 mg, 1.25 mmol), 3-aminopiperidine-2,6-dione hydrogen chloride (204 mg, 1.24 mmol) and sodium acetate (110 mg, 1.34 mmol) in acetic acid (10 mL). The product is a white solid (200 mg, 56% yield): mp 263.0-265.0 °C; ^{1}H NMR (DMSO-d_6) δ 2.01-2.07 (m, 1H, CH*H*), 2.50-2.89 (m, 9H, C*H*$_3$, CH*H*, C*H*$_2$), 5.10 (dd, *J* = 5.1, 12.4 Hz, 1H, NC*H*), 7.52 (s, 2H, Ar), 11.12 (br s, 1H, N*H*); ^{13}C NMR (DMSO-d_6) δ 16.82, 22.02, 30.97, 48.59, 128.01, 135.04, 136.58, 167.68, 169.98, 172.83.

EXAMPLE 6

2-(2,6-Dioxo(3-piperidyl))-4-ethylisoindoline-1,3-dione

2-(2,6-Dioxo(3-piperidyl))-4-ethylisoindoline-1,3-dione was prepared by the procedure of Example 1 from 3-ethylphthalic anhydride (0.860 g, 4.89 mmol), 3-aminopiperidine-2,6-dione hydrogen chloride (0.803 g, 4.88 mmol) and sodium acetate (0.420 g, 5.12 mmol) in acetic acid (10 mL). The product was a white solid (1.06 g, 76 % yield); mp, 235.0-236.5 °C; ^{1}H NMR (DMSO-d_6) δ 1.22 (t, *J* = 7.4 Hz, 3H, C*H*$_3$), 2.04-2.10 (m, 1H, CH*H*), 2.47-2.63 (m, 2H, C*H*$_2$), 2.83-2.98 (m, 1H, CH*H*), 3.07 (q, *J* = 7.5 Hz, 2H, C*H*$_2$), 5.13 (dd, *J* = 5.4, 12.5 Hz, 1H, NC*H*), 7.70-7.82 (m, 3H, Ar), 11.13 (br s, 1H, N*H*); ^{13}C NMR (DMSO-d_6) δ 14.84, 21.95, 23.69, 30.90, 48.77, 121.09, 127.26, 131.76, 134.63, 135.39, 143.87, 166.99, 167.58, 169.85, 172.72; Anal Calcd for $C_{15}H_{14}N_2O_4$: C, 62.93; H, 4.93; N, 9.79. Found: C, 62.74; H, 4.84; N, 9.54.

EXAMPLE 7

4-Methoxy-2-(2,6-dioxo(3-piperidyl))isoindoline-1,3-dione

4-Methoxy-2-(2,6-dioxo(3-piperidyl))isoindoline-1,3-dione was prepared by the procedure of Example 1 from 3-methoxyphthalic anhydride (1.0 g, 5.6 mmol) {Rao. A.V.R. et al, Indian J. Chem. 1981, 20 (B), 248}, 3-aminopiperidine-2,6-dione hydrogen chloride (0.92 g, 5.6 mmol) and sodium acetate (0.48 g, 6.0 mmol) in acetic acid (20 mL). The product was a white solid (0.44 g, 27 % yield); mp, 281.5-282.5 °C; ^{1}H NMR (DMSO-d_6) δ 2.00-2.08 (m, 1H, CH*H*), 2.56-2.62 (m, 2H, CH_2), 2.82-2.91 (m, 1H, CH*H*), 3.97 (s, 3H, CH_3), 5.08 (dd, *J* = 5.3, 12.8 Hz, 1H, NC*H*), 7.46 (d, *J* = 7.2 Hz, 1H, Ar), 7.52 (d, *J* = 8.5 Hz, 1H, Ar), 7.84 (d, *J* = 7.8 Hz, 1H, Ar), 11.10 (br s, 1H, N*H*); ^{13}C NMR (DMSO-d_6) δ 21.97, 30.92, 48.73, 56.33, 115.24, 116.11, 119.01, 133.19, 137.15, 156.49, 165.37, 166.84, 169.94, 172.79; Anal Calcd for $C_{14}H_{12}N_2O_5$: C, 58.33; H, 4.20; N, 9.72. Found: C, 58.23; H, 3.90; N, 9.53.

EXAMPLE 8

4-Dimethylamino-2-(2,6-dioxo(3-piperidyl))isoindoline-1,3-dione

4-Dimethylamino-2-(2,6-dioxo(3-piperidyl))isoindoline-1,3-dione was prepared by the procedure of Example 1 from 3-dimethylaminophthalic anhydride (1.34 g, 7.0 mmol), 3-aminopiperidine-2,6-dione hydrogen chloride (1.15 g, 7.0 mmol) and sodium acetate (0.60 g, 7.3 mmol) in acetic acid (20 mL). The product was a yellow solid (1.59 g, 75 % yield); mp, 214.5-216.5 °C; ^{1}H NMR (DMSO-d_6) δ 1.98-2.09 (m, 1H, CH*H*), 2.49-2.62 (m, 2H, CH_2), 2.81-2.95 (m, 1H, CH*H*), 3.04 (s, 6H, CH_3), 5.08 (dd, *J* = 5.5, 12.7 Hz, 1H, NC*H*), 7.23 (d, *J* = 6.6 Hz, 1H, Ar), 7.26 (d, *J* = 8.1 Hz, 1H, Ar), 7.63 (dd, *J* = 6.9, 8.6 Hz, 1H, Ar), 11.09 (br s, 1H, N*H*); ^{13}C NMR (DMSO-d_6) δ 22.10, 30.96, 42.95, 48.77, 112.99, 113.41, 122.59, 133.90, 135.22, 149.88, 166.29, 167.13, 170.06, 172.83; Anal Calcd for $C_{15}H_{15}N_3O_4$: C, 59.80; H, 5.02; N, 13.95. Found: C, 59.60; H, 4.94; N, 13.80.

EXAMPLE 9

2-(2,6-Dioxo(3-piperidyl))-4-chloroisoindoline-1,3-dione

2-(2,6-Dioxo(3-piperidyl))-4-chloroisoindoline-1,3-dione was prepared by the procedure of Example 1 from 3-chlorophthalic anhydride (0.40 g, 2.2 mmol), 3-aminopiperidine-2,6-dione hydrogen chloride (0.36 g, 2.2 mmol) and sodium acetate (0.19 g, 2.4 mmol) in acetic acid (10 mL). The product was a white solid (0.44 g, 69 % yield); mp, 290.0-291.5 °C; ^{1}H NMR (DMSO-d_6) δ 2.05-2.11 (m, 1H, CH*H*), 2.49-2.64 (m, 2H, CH_2), 2.64-2.92 (m, 1H, CH*H*), 5.17 (dd, *J* = 5.2, 12.7 Hz, 1H, NC*H*), 7.86-7.94 (m, 3H, Ar), 11.17 (br s, 1H, N*H*); ^{13}C NMR (DMSO-d_6) δ 21.83, 30.91, 49.12, 122.41, 126.94, 129.84, 133.52, 136.11, 136.39, 164.77, 165.76, 169.73, 172.77; Anal Calcd for $C_{13}H_9N_2O_4Cl$: C, 53.35; H, 3.10; N, 9.57; Cl, 12.11. Found: C, 53.37; H, 2.94; N, 9.30, Cl, 11.97.

EXAMPLE 10

4-Methyl-2-(2,6-dioxo-3-methyl-(3-piperidyl))isoindoline-1,3-dione

4-Methyl-2-(2,6-dioxo-3-methyl-(3-piperidyl))isoindoline-1,3-dione was prepared by the procedure of Example 1 from 3-methylphthalic anhydride (0.27 g, 1.7 mmol), 3-amino-3-methylpiperidine-2,6-dione hydrogen chloride (0.30 g, 1.7 mmol) and sodium acetate (0.15 g, 1.8 mmol) in acetic acid (10 mL). The product was a white solid (0.13 g, 27 % yield); mp, 248.0-250.0 °C; ^{1}H NMR (DMSO-d_6) δ 1.89 (s, 3H, CH_3), 2.01-2.08 (m, 1H, CH*H*), 2.49-2.70 (m, 3H, CH*H*, CH_2), 2.55 (s, 3H, CH_3), 7.62-7.74 (m, 3H, Ar), 10.99 (br s, 1H, N*H*); ^{13}C NMR (DMSO-d_6) δ 17.0, 21.0, 28.6, 29.1, 58.6, 120.7, 127.5, 131.5, 134.2, 136.8, 137.2, 167.7, 168.6, 172.1, 172.3; Anal. Calcd. for $C_{15}H_{14}N_2O_4$ + 0.3 H_2O: C, 61.77; H, 5.05; N, 9.60. Found: C, 62.05; H, 4.94; N, 9.20.

EXAMPLE 11

Tablets, each containing 50 mg of 1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-methyl-isoindoline, can be prepared in the following manner:

Constituents (for 1000 tablets)

1-oxo-2-(2,6-dioxo-piperidin-3-yl)-4-methyl-isoindoline	50.0 g
lactose	50.7 g
wheat starch	7.5 g
polyethylene glycol 6000	5.0 g
talc	5.0 g
magnesium stearate	1.8 g
demineralized water	q.s.

The solid ingredients are first forced through a sieve of 0.6 mm mesh width. The active ingredient, lactose, talc, magnesium stearate and half of the starch then are mixed. The other half of the starch is suspended in 40 mL of water and this suspension is added to a boiling solution of the polyethylene glycol in 100 mL of water. The resulting paste is added to the pulverulent substances and the mixture is granulated, if necessary with the addition of water. The granulate is dried overnight at 35°C, forced through a sieve of 1.2 mm mesh width and compressed to form tablets of approximately 6 mm diameter which are concave on both sides.

EXAMPLE 12

Gelatin dry-filled capsules, each containing 100 mg of 1,3-dioxo-2-(2,6-dioxo-piperidin-3-yl)-4-methylisoindoline, can be prepared in the following manner:

Composition (for 1000 capsules)

1,3-dioxo-2-(2,6-dioxo-piperidin-3-yl)-4-methyl-isoindoline	100.0 g
microcrystalline cellulose	30.0 g
sodium lauryl sulfate	2.0 g

magnesium stearate ..... 8.0 g

The sodium lauryl sulfate is sieved into the 1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-methylisoindoline through a sieve of 0.2 mm mesh width and the two components are intimately mixed for 10 minutes. The microcrystalline cellulose is then added through a sieve of 0.9 mm mesh width and the whole is again intimately mixed for 10 minutes. Finally, the magnesium stearate is added through a sieve of 0.8 mm width and, after mixing for a further 3 minutes, the mixture is introduced in portions of 140 mg each into size 0 (elongated) gelatin dry-fill capsules.

EXAMPLE 13

A 0.2% injection or infusion solution can be prepared, for example, in the following manner:

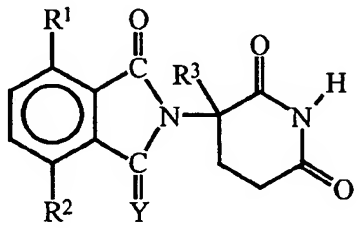
1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4,7-dimethylisoindoline ..... 5.0 g
sodium chloride..... 22.5 g
phosphate buffer pH 7.4.....300.0 g
demineralized water ..... to 2500.0 mL

1-Dioxo-2-(2,6-dioxopiperidin-3-yl)-4,7-dimethylisoindoline is dissolved in 1000 mL of water and filtered through a microfilter. The buffer solution is added and the whole is made up to 2500 mL with water. To prepare dosage unit forms, portions of 1.0 or 2.5 mL each are introduced into glass ampoules (each containing respectively 2.0 or 5.0 mg of imide).

What is claimed is:

1. A compound selected from the group consisting of

(*a*) a 2-(2,6-dioxopiperidin-3-yl)-isoindoline of the formula:



I.

in which

Y is oxygen or H_2,

one of R^1 and R^2 is halo, alkyl, alkoxy, alkylamino, dialkylamino, cyano, or carbamoyl,

the other of R^1 and R^2 is independently hydrogen, halo, alkyl, alkoxy, alkylamino, dialkylamino, cyano, or carbamoyl, and

R^3 is hydrogen, alkyl, or benzyl, and

(*b*) the acid addition salts of said 2-(2,6-dioxopiperidin-3-yl)-isoindolines which contain a nitrogen atom capable of being protonated.

2. The compound according to claim 1, in which Y is oxygen.

3. The compound according to claim 1, in which Y is H_2.

4. The compound according to claim 1, in which R^1 and R^3 are hydrogen.

5. The compound according to claim 4 in which R^2 is methyl, ethyl, chloro, or methoxy.

6. The compound according to claim 1, in which R^2 and R^3 are methyl and R^1 is hydrogen.

7. The compound according to claim 1, which is substantially chirally pure (S)-1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-methylisoindoline, substantially chirally pure (R)-1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-methylisoindoline, or mixtures thereof.

8. The compound according to claim 1, which is substantially chirally pure (S)-1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-ethylisoindoline, substantially chirally pure (R)-1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4-ethylisoindoline, or mixtures thereof.

9. The compound according to claim 1, which is substantially chirally pure (S)-1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4,7-dimethylisoindoline, substantially chirally pure (R)-1,3-dioxo-2-(2,6-dioxopiperidin-3-yl)-4,7-dimethylisoindoline, or mixtures thereof.

10. The compound according to claim 1, which is substantially chirally pure (S)-1,3-dioxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4-methylisoindoline, substantially chirally pure (R)-1,3-dioxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4-methylisoindoline, or mixtures thereof.

11. The compound according to claim 1, which is substantially chirally pure (S)-1,3-dioxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)- 4,7-dimethylisoindoline, substantially chirally pure (R)-1,3-dioxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)- 4,7-dimethylisoindoline, ord mixtures thereof.

12. The compound according to claim 1, which is substantially chirally pure (S)-1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-methylisoindoline, substantially chirally pure (R)-1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-methylisoindoline, or mixtures thereof.

13. The compound according to claim 1, which is substantially chirally pure (S)-1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-ethylisoindoline, substantially chirally pure (R)-1-oxo-2-(2,6-dioxopiperidin-3-yl)-4-ethylisoindoline, or mixtures thereof.

14. The compound according to claim 1, which is substantially chirally pure (S)-1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,7-dimethylisoindoline, substantially chirally pure (R)-1-oxo-2-(2,6-dioxopiperidin-3-yl)-4,7-dimethylisoindoline, or mixtures thereof.

15. The compound according to claim 1, which is substantially chirally pure (S)-1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4-methylisoindoline, substantially chirally pure (R)-1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)-4-methylisoindoline, or mixtures thereof.

16. The compound according to claim 1, which is substantially chirally pure (S)-1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)- 4,7-dimethylisoindoline, substantially chirally pure (R)-1-oxo-2-(2,6-dioxo-3-methylpiperidin-3-yl)- 4,7-dimethylisoindoline, or mixtures thereof.

17. A method of reducing undesirable levels of inflammatory cytokines in a mammal which comprises administering thereto an effective amount of a compound according to claim 1.

18. A pharmaceutical composition comprising a quantity of a compound according to claim 1, sufficient upon administration in a single or multiple dose regimen to reduce levels of inflammatory cytokines in a mammal in combination with a carrier.

19. A method of treating inflammation in a mammal which comprises administering thereto an effective amount of a compound according to claim 1.

20. A method of treating autoimmune diseases in a mammal which comprises administering thereto an effective amount of a compound according to claim 1.

21. A method of treating in a mammal a disease selected from the group consisting of arthritis, rheumatoid arthritis, inflammatory bowel disease, Crohn's disease, aphthous ulcers, cachexia, graft versus host disease, asthma, adult respiratory distress syndrome, and acquired immune deficiency syndrome, which comprises administering thereto an effective amount of a compound according to claim 1.

22. A method of treating cancer in a mammal which comprises administering thereto an effective amount of a compound according to claim 1.

23. A method of treating undesirable angiogenesis in a mammal which comprises administering thereto an effective amount of a compound according to claim 1.

24. A method of reducing or inhibiting undesirable levels of TNFα in a mammal which comprises administering thereto an effective amount of a compound according to claim 1.

25. A method of treating inflammatory diseases in a mammal which comprises administering thereto an effective amount of a compound according to claim 1.

26. The compound according to claim 1, which is substantially chirally pure (S)-isomer of a 2-(2,6-dioxopiperidin-3-yl)-isoindoline, a substantially chirally pure (R)-isomer of a 2-(2,6-dioxopiperidin-3-yl)-isoindoline, or mixtures thereof.

27. A method of reducing or inhibiting undesirable levels of IL-1 in a mammal which comprises administering thereto an effective amount of a compound according to claim 1.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/05562

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D401/04 A61K31/445

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GB 1 075 420 A (CHEMIE GRUNENTHAL G.M.B.H.) 12 July 1967 see example 31 ---	1,2
X	WO 98 03502 A (CELGENE CORPORATION) 29 January 1998 see claims 1,5-7 ---	1-4,18
P,X	MIYACHI H. ET AL.: "Tumor necrosis factor-alpha production enhancing activity of substituted 3'-methylthalidomide: Influence of substituents at the phthaloyl moiety on the activity and stereoselectivity" CHEMICAL & PHARMACEUTICAL BULLETIN, vol. 46, no. 7, July 1998, pages 1165-1168, XP002107774 see compounds (R)-9d and (S)-9d ---	1,2,4,18
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
30 June 1999	09. 07. 99

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/05562

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 54170 A (CELGENE CORPORATION) 3 December 1998 see page 10, line 3 - line 23; claims 6,10,12	1-4,18
Y	WO 92 14455 A (THE ROCKEFELLER UNIVERSITY) 3 September 1992 see claims 1,2,6,11,12	1-16,18, 26
Y	EP 0 688 771 A (GRÜNENTHAL GMBH) 27 December 1995 see claims 1,2,5,6	1-16,18, 26
Y	NIWAYAMA S. ET AL.: "Potent inhibition of tumor necrosis factor-.alpha. production by tetrafluorothalidomide and tetrafluorophthalimides" JOURNAL OF MEDICINAL CHEMISTRY, vol. 39, no. 16, 1 January 1996, pages 3044-3045, XP002048231 see the whole document	1-16,18, 26
Y	US 5 635 517 A (MULLER G.W. ET AL.) 3 June 1997 see the whole document	1-16,18, 26

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/05562

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 17,19-25,27
because they relate to subject matter not required to be searched by this Authority, namely:

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/05562

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